


Effects of corn particle size on growth performance, gastrointestinal development, carcass indices and intestinal microbiota of broilers

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ABSTRACT This experiment was conducted to investigate the effects of different corn particle sizes on growth performance, gastrointestinal development, carcass processing yields and intestinal microbiota of caged broilers. One-day-old Ross 308 broilers were randomly divided into 8 treatments with 10 replicates per treatment and 30 birds per replicate pen. The experiment lasted 37 d. Feed and water were provided ad libitum. The results showed as follows: birds fed diets with the FG corn between d 1 and 13 and CG corn between d14 to 37 had increased body weight, daily gain, and feed intake ($P < 0.05$). Birds fed diets with CG corn between d 24 to 37 had a heavier relative weight of gizzard at d 38 ($P < 0.05$). Birds fed diets with FG corn from d 1 to

13 and the CG corn from d 14 to 37 had a higher carcass yield and a relative thigh weight at d 38 ($P < 0.05$). The intestinal microbiota was significantly affected by different corn particle sizes. The relative abundance of *Lactobacillaceae* was significantly decreased, whereas that of *Peptostreptococcaceae* was increased ($P < 0.05$) in birds fed with the CG corn between d1 to 37. The relative abundance of *Acinetobacter* was significantly increased in birds fed the FG corn between d1 to 37 ($P < 0.05$). In conclusion, the use of FG corn in the starter phase and CG corn in the grower and finisher phases was beneficial to growth performance, gastrointestinal development and intestinal microbial structure of broilers reared in cages.

Key words: corn particle size, broiler, growth performance, carcass yield, intestinal microflora

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INTRODUCTION

Feed processing is an essential step in production performance and economic benefit of livestock (Putra et al., 2018). The grinding degree of grain not only affects the processing quality of feed products, but also influences feed nutritional value, broiler growth performance and gastro-intestinal function (Abdollahi et al., 2019). Studies have shown that dietary coarse ground grain effectively improved feed conversion ratio (FCR) of broilers and the utilization efficiency of feed nutrients for broilers (Xu et al., 2017). Kheravii et al. (2017) found that corn coarse particles reduced the feed to gain ratio of broiler by 1.4%. Amerah et al. (2007) reported coarse particles improved broiler FCR from 1.621 to 1.575 from d 1 to 21. Birds struggle to consume too large or too fine particles depending on their age as they are limited by their

beak size (Moran, 1982). Thus, Healy et al. (2010) showed that fine grinding was conducive to improving broiler performance during d 1 to 21. The inclusion of 50% coarsely ground ingredients in broiler diets increased carcass yield and reduced the ratio of feed to gain at 49 d of age (Xu et al., 2017). The hypothesis was that feeding birds feeds with different particle sizes at different phases of growth may maximize their performance potential and enhance their gut health. For instance, feeding coarsely-ground corn in the later phase may produce beneficial effects to birds gastrointestinal development. The current study evaluated whether finely ground (FG) or coarsely ground (CG) corn would have influence growth performance, carcass yields, gastrointestinal development and intestinal microbiota at different phases of the broiler growth cycle.

MATERIALS AND METHODS

The study was approved by the Animal Care and Experiment Committee of New Hope Liuhe Corporation. The management and husbandry of the birds strictly followed

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Table 1. Experiment design.

Items		Starter (d 1–13)	Grower (d 14–23)	Finisher (d 24–37)
Treatment 1	F/F/F	Fine Ground	Fine Ground	Fine Ground
Treatment 2	C/C/C	Coarse Ground	Coarse Ground	Coarse Ground
Treatment 3	F/F/C	Fine Ground	Fine Ground	Coarse Ground
Treatment 4	F/C/C	Fine Ground	Coarse Ground	Coarse Ground
Treatment 5	C/C/F	Coarse Ground	Coarse Ground	Fine Ground
Treatment 6	C/F/F	Coarse Ground	Fine Ground	Fine Ground
Treatment 7	C/F/C	Coarse Ground	Fine Ground	Coarse Ground
Treatment 8	F/C/F	Fine Ground	Coarse Ground	Fine Ground

C, coarse ground; F, fine ground.

Table 2. Measured corn particle sizes in starter, grower and finisher diets for birds reared in cages.

Fine Ground/ μm (d 1–37)	Coarse Ground / μm		
	Starter (d 1–13)	Grower (d 14–23)	Finisher (d 24–37)
485 \pm 13.86	1693 \pm 36.93	2310 \pm 121.92	2409 \pm 107.57

F corn maintained the same particle size regardless of growth phase or treatment, C corn varied in particle size from 1693 μm for the starter, 2310 μm for the grower, and 2409 μm for finisher period.

the Chinese government's regulations on animal welfare. This research on live animals met the guidelines approved by the institutional animal care and use committee (IACUC).

Experimental Design

A total of twenty four hundred 1-day-old male Ross 308 broilers were divided into 8 treatments (Treatment 1–8) with 10 replicates in each treatment and 30 broilers in each replicate (cage). Treatment 1 was the control group, which was fed diets containing finely ground, designated as the F corn with a grind size of 485 μm throughout the three phases of the study (Starter: d 1–13; Grower: d 14–23; and Finisher: d 24–37), abbreviated as F/F/F. While the F corn maintained the same particle size regardless of growth phase or treatment, the coarsely ground, designated as the C corn varied in particle size from 1,693 μm for the starter, 2,310 μm for the grower and 2,409 μm for finisher period. The fine particle size was obtained using a hammer mill with a 2 mm screen, whereas the coarse corn particle size was obtained using a hammer-mill without the screen. The full experimental design is given in Table 1. The geometric mean diameter (GMD) of corn particle size was determined according to the GB/T 6971-2007. Particle size was determined using a 14-sieve stack with sieve numbers 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270 and a pan. Approximately 100 g of samples were used to sift for 10 min. Geometric mean particle size by mass and the geometric standard deviation of particle diameter by mass were determined using the quantity of material. The other main feed ingredients and feed additives were in the same particle size.

The measured corn particle sizes in each phase were shown in Table 2.

Diets and Bird Management

Diets were formulated to meet the nutrient recommendations for Ross 308 (Aviagen, 2014). The feeding program consisted of a starter (d 1–13), grower (d 14–23), and finisher phase (d 24–37). Diets were crumbled for the starter phase, and pelleted for both grower and finisher phases. The ingredients and nutrient compositions of the experimental diets were shown in Table 3. The experimental broilers were raised in cages. The broiler houses had 3 vertical tiers of metal cages. The stocking density of rearing was 12.5 birds/ m^2 . The brooding temperature was maintained at 33°C for the first day and was gradually decreased by 2°C per wk until 21°C and maintained at that level thereafter. Feed and water were provided ad libitum. The lighting, relative humidity, and temperature followed the Ross 308 guide lines (Aviagen, 2014).

Sample and Data Collection

Growth Performance Body weight (BW) and feed intake (FI) by pen were recorded on d 13, 23, and 37, and mortality was recorded daily. Average daily feed intake (ADFI), and FCR were calculated for starter, grower, finisher and overall periods.

Carcass Yields At d 38, 10 broilers with similar BW (1 bird per cage with a body weight close to the cage average weight) were selected from each treatment, weighed and killed by exsanguinations after CO₂ stunning. Birds were placed in a closed box, which was ducted to a CO₂. Birds were exposed to CO₂ for 3 to 4 min until they were completely asphyxiated. Then breast muscle, thigh, swing, abdominal fat were taken for weighing.

Digestive Organ Development At d 14, 24, and 38, 10 birds with similar BW (1 bird per cage with a body weight close to the cage average weight) from each treatment were selected and slaughtered, respectively.

Table 3. Composition and nutrient levels of basal diets (air-dry basis, %).

	Starter (d 1–13)	Grower (d 14–23)	Finisher (d 24–37)
Corn	59.43	62.71	63.85
Soybean meal	27.80	21.60	16.00
Peanut meal	4.00	5.00	6.00
Corn protein powder	2.00	3.00	4.00
Corn DDGS	2.00	3.00	4.00
Soybean oil	0.30	0.63	2.34
CaHPO ₄	1.63	1.18	0.84
Limestone	1.22	1.12	1.09
NaCl	0.30	0.30	0.30
L-Lys-H ₂ SO ₄	0.49	0.64	0.76
DL-Met(98.5%)	0.23	0.22	0.22
Choline chloride	0.10	0.10	0.10
Premix ¹	0.50	0.50	0.50
Total	100.00	100.00	100.00
Nutrients levels			
Crude Protein	22.00	21.00	20.00
ME MJ/kg	11.81	12.23	12.85
Ca	0.85	0.75	0.65
Total P	0.60	0.53	0.45

¹The premix provides following per kg diet: Cu 8 mg, Zn 75 mg, Fe 80 mg, Mn 100 mg, Se 0.15 mg, I 0.35 m, VA 9000 IU, VD₃ 2000 IU, VE 11 IU, VK1.0 mg, VB₁1.2mg, VB₂ 5.8 mg, niacin 66mg, pantothenic acid 12 mg, VB₆ 2.6 mg, biotin0.10 mg, folic acid 0.7 mg, VB₁₂ 0.010 mg. Abbreviations: DDGS, distillers dried grains with solubles; ME, metabolizable energy. All the values are calculated.

Proventriculus and gizzard were taken for weighing. The length of duodenum, jejunum and ileum was measured, and each segment of intestinal tract was weighed after the digesta was taken. The relative weight and length of the intestine were calculated as follows:

Relative intestinal weight (%)

$$= \text{intestinal weight (g)} / \text{body weight (g)} \times 100;$$

Intestinal weight per length(g/cm)

$$= \text{intestinal weight (g)} / \text{intestinal length (cm)}.$$

Ileal Digesta At d 38, 10 broilers from 4 treatments: F/F/F, C/C/C, F/C/C, and C/F/F were slaughtered, the ileal digesta was collected and stored at -80°C for analysis of the intestinal microbiota.

- (1) DNA extraction: DNA extraction and PCR amplification were carried out using fecal microbes DNA extraction kit (QIAamp Fast DNA Stool Mini Kit, Qiagen company, Germany) for the profiling of the ileal microbial DNA. The samples were diluted to 1 ng/ μL with sterile water, and the diluted DNA was used as a template. The universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') in the V4 region of 16SrDNA gene were used to amplify bacterial DNA.
- (2) Preparation of PCR Products: The same amount of PCR product was mixed according to the PCR product concentration. After full mixing, the PCR products were detected by 2% agarose gel electrophoresis. The

target bands were recovered using the gel recovery kit provided by QIAGEN Company.

- (3) Library Construction and Sequencing: The TRAU-SEQ DNA PCR-Free sample Preparation Kit was used for library construction. The constructed libraries were quantitated by Qubit and Q-PCR. After the libraries were qualified, computer sequencing was performed using Hiseq2500PE250.
- (4) Sequence Data Processing: Using the Uparse software (Uparsev7.0.1001) for all the samples of Effective Tags clustering. The default, with 97% of the consistency will become the sequence clustering OTUs (Operational Taxonomic Units). Subsequent diversity analysis was based on the data after homogenization. Wien plots, dilution plots, coverage index plots, PCA plots, PCOA plots, and ANOSIM analyses were drawn using R software (Version2.15.3). Metagenomic function prediction analysis was performed using Picrust software.

Statistical Analysis

Experimental data were analyzed by univariate and multivariate ANOVA using SPSS18.0 statistical software, and Duncan's multiple range test was used for multiple comparisons. The model included the main effects of particle size, days of age and their interaction. $P < 0.05$ was used as the criterion of significant difference.

RESULTS

Growth Performance

Effects of corn size on growth performance of broilers are shown in Table 4.

Table 4. Different particle sizes of corn on growth performance of broilers between d 1 and 37.

Items	Group									SEM	P-value
	F/F/F	C/C/C	F/F/C	F/C/C	C/C/F	C/F/F	C/F/C	F/C/F			
1 BW/g	43.4	43.6	43.4	43.6	43.6	43.6	43.6	43.8	0.66	0.080	
37 d BW/g	2737 ^{cd}	2780 ^{abc}	2810 ^{ab}	2831 ^a	2800 ^{ab}	2699 ^d	2791 ^{ab}	2770 ^{bc}	7.20	<0.001	
FCR	1.477	1.487	1.478	1.478	1.480	1.493	1.482	1.480	0.002	0.380	
ADFI/g	108.2 ^{cd}	110.0 ^{abc}	110.5 ^{ab}	111.3 ^a	110.2 ^{ab}	107.4 ^d	110.0 ^{abc}	109.1 ^{bcd}	0.25	<0.001	
Survival rate/%	98.0	99.0	99.3	98.3	99.3	97.9	99.0	98.3	0.23	0.612	
Main effect	Starter (d 1–13)		Grower (d 14–23)		Finisher (d 24–37)		P-value				
	Fine	Coarse	Fine	Coarse	Fine	Coarse	Starter	Grower	Finisher	Starter × Grower × Finisher	
37 d BW/g	2,787	2,768	2,759 ^b	2,795 ^a	2,752 ^b	2,803 ^a	0.075	0.007	<0.001	0.017	
FCR	1.478	1.485	1.482	1.481	1.482	1.481	0.091	0.495	0.513	0.469	
ADFI/g	109.8	109.4	109.1 ^b	110.1 ^a	108.7 ^b	110.5 ^a	0.317	0.007	<0.001	0.080	
Survival rate/%	98.5	98.8	98.6	98.7	98.4	98.9	0.366	0.709	0.281	0.491	

Abbreviations: BW, body weight; ADFI, average daily feed intake; FCR, feed conversion ratio.

^{a-d}Within a line, numbers with different superscripts differ statistically at $P < 0.05$.

Main effects: corn particle size had no significant effect on the growth performance during the starter period ($P > 0.05$). However, coarse corn significantly improved BW and ADFI during the grower and finisher periods compared with the fine corn treatments ($P < 0.05$). There were significant interactions on BW due to corn particle size and bird age ($P < 0.05$). Treatment 4, where birds were fed a diet containing fine corn at starter followed by coarse corn at grower and finisher (F/C/C group) had the best overall performance including final BW and ADFI ($P < 0.05$). Treatment 2, birds in the C/C/C group had a better final body weight and FCR, compared with the F/F/F group, but the difference was not significant ($P > 0.05$).

Carcass Yields

Effects of corn particle size on carcass yields of broilers were shown in Table 5.

Main effect: Coarse corn significantly increased relative weights of thigh and abdominal fat at d 24 ($P < 0.05$). Coarse corn also increased the overall carcass yield, the relative weights of thighs and wings at d 38 ($P < 0.05$). There was a significant particle size × age on

wing weight ($P < 0.05$). Compared with the control (F/F/F group), the F/C/C group had increased carcass yield, thigh weight and wing weight at d 38 ($P < 0.05$). The relative weight of the abdominal fat in the F/F/F group was significantly lower than that in the CCC group at d 38 ($P < 0.05$).

Gastrointestinal Development

Effects of different corn particle sizes on the gastrointestinal development of broilers are shown in Table 6.

Main effects: In general, fine corn improved the jejunal weight relative to length than coarse corn at d14 ($P < 0.05$). Coarse corn increased the relative weight of the proventriculus, jejunum and ileum at d 24 ($P < 0.05$) and the relative weight of the gizzard at d 38 ($P < 0.05$). While fine corn increased the weight of the duodenum and jejunum relative to their lengths as well as the relative weights of the proventriculus and small intestine at d 38 ($P < 0.05$). There were significant particle size × age interactions for the relative weights of the gizzard, proventriculus and duodenum at d 38 ($P < 0.05$).

Birds in the C/C/C, F/F/C, and F/C/C groups had higher gizzard relative weights than other groups

Table 5. Effect of particle size on carcass yields of broiler.

Items	Group									SEM	P-value
	F/F/F	C/C/C	F/F/C	F/C/C	C/C/F	C/F/F	C/F/C	F/C/F			
Carcass yield	72.53 ^c	74.51 ^{ab}	74.91 ^a	74.96 ^a	74.34 ^{abc}	73.14 ^{abc}	73.21 ^{abc}	72.75 ^{bc}	0.23	0.012	
Breast yield	21.51	21.68	21.36	20.82	21.24	21.38	20.69	20.78	0.15	0.584	
Thigh yield	23.48 ^c	24.54 ^{ab}	24.65 ^a	24.83 ^a	24.18 ^{abc}	23.57 ^{bc}	23.66 ^{bc}	23.9 ^{abc}	0.12	0.012	
Wing yield	7.23 ^c	7.28 ^c	7.55 ^{abc}	7.69 ^{ab}	7.47 ^{abc}	7.33 ^{bc}	7.74 ^a	7.48 ^{abc}	0.04	0.021	
Abdominal fat	1.44 ^{bc}	1.74 ^a	1.24 ^c	1.61 ^{ab}	1.49 ^{bc}	1.36 ^{bc}	1.51 ^{bc}	1.64 ^{ab}	0.04	0.006	
Main effect	14 d		24 d		38 d		P-value				
	Fine	Coarse	Fine	Coarse	Fine	Coarse	Starter	Grower	Finisher	Starter × Grower × Finisher	
Carcass yield	73.79	73.8	73.45	74.14	73.19 ^b	74.4 ^a	0.981	0.102	0.005	0.082	
Breast yield	21.12	21.25	21.24	21.13	21.23	21.14	0.661	0.713	0.757	0.276	
Thigh yield	24.22	23.99	23.84 ^b	24.36 ^a	23.78 ^b	24.42 ^a	0.304	0.021	0.005	0.313	
Wing yield	7.49	7.46	7.46	7.48	7.38 ^b	7.57 ^a	0.716	0.812	0.025	0.020	
Abdominal fat	1.48	1.51	1.39 ^b	1.61 ^a	1.48	1.51	0.572	0.001	0.557	0.153	

^{a-c}Within a line, numbers with different superscripts differ statistically at $P < 0.05$.

Table 6. Different particle sizes of corn on development of broiler digestive organs at d 38.

Items	Group								SEM	P-value	
	F/F/F	C/C/C	F/F/C	F/C/C	C/C/F	C/F/F	C/F/C	F/C/F			
Relative weight of gizzard %	0.885 ^c	1.271 ^a	1.16 ^b	1.111 ^b	0.871 ^c	0.880 ^c	1.049 ^b	0.849 ^c	0.021	<0.001	
Relative weight of proventriculus %	0.482 ^a	0.318 ^c	0.353 ^{bc}	0.317 ^c	0.339 ^{bc}	0.388 ^b	0.324 ^{bc}	0.315 ^c	0.011	<0.001	
Weight of unit length g/cm	Duodenum	0.532 ^{ab}	0.477 ^{bc}	0.479 ^{bc}	0.465 ^c	0.482 ^{bc}	0.519 ^{abc}	0.456 ^c	0.554 ^a	0.008	0.012
	Jejunum	0.393 ^a	0.324 ^c	0.358 ^{abc}	0.366 ^{ab}	0.350 ^{bc}	0.373 ^{ab}	0.352 ^{bc}	0.372 ^{ab}	0.004	0.006
	Ileum	0.311	0.290	0.297	0.296	0.290	0.289	0.280	0.281	0.004	0.450
Relative weight of intestine %	Duodenum	0.654 ^a	0.544 ^{bc}	0.534 ^c	0.481 ^c	0.540 ^{bc}	0.614 ^{ab}	0.489 ^c	0.614 ^{ab}	0.011	<0.001
	Jejunum	1.073 ^a	0.881 ^c	0.934 ^{bc}	0.935 ^{bc}	0.936 ^{bc}	1.114 ^a	0.917 ^{bc}	1.027 ^{ab}	0.016	<0.001
	Ileum	0.906 ^a	0.776 ^b	0.777 ^b	0.746 ^b	0.775 ^b	0.896 ^a	0.775 ^b	0.813 ^{ab}	0.012	0.002

Main effect	14d		24d		38d		P-value				
	Fine	Coarse	Fine	Coarse	Fine	Coarse	Starter	Grower	Finisher	Starter×Grower×Finisher	
Relative weight of gizzard %	1.001	1.018	0.993	1.026	0.871 ^b	1.148 ^a	0.552	0.250	<0.001	0.005	
Relative weight of proventriculus %	0.367	0.342	0.387 ^a	0.322 ^b	0.381 ^a	0.328 ^b	0.107	<0.001	0.001	0.004	
Weight of unit length g/cm	Duodenum	0.507	0.484	0.496	0.495	0.522 ^a	0.469 ^b	0.109	0.893	0.001	0.365
	Jejunum	0.372 ^a	0.350 ^b	0.369	0.353	0.372 ^a	0.350 ^b	0.007	0.053	0.009	0.556
	Ileum	0.296	0.287	0.294	0.289	0.293	0.291	0.206	0.480	0.777	0.333
Relative weight of intestine %	Duodenum	0.571	0.547	0.573	0.545	0.606 ^a	0.512 ^b	0.175	0.114	<0.001	0.029
	Jejunum	0.992	0.962	1.009 ^a	0.945 ^b	1.038 ^a	0.917 ^b	0.281	0.023	<0.001	0.216
	Ileum	0.811	0.806	0.839 ^a	0.778 ^b	0.847 ^a	0.769 ^b	0.825	0.009	0.001	0.267

^{a-c}Within a line, numbers with different superscripts differ statistically at $P < 0.05$.

($P < 0.05$), but had lower duodenum and jejunum weights relative to their lengths and lower relative weights of the duodenum, jejunum and ileum at d 38 ($P < 0.05$).

Ileal Microflora

Effects of different particle sizes on the ileal microbiota of broilers are shown in Figure 1. A 97% sequence similarity was used as the threshold of OTUs, and a total of 2,724 OTUs were obtained, among which 1162 OTUs were shared by 4 treatment groups, that is, F/F/F, C/C/C, F/C/C, and F/C/C. The unique OTUs for the 4 groups were 46, 70, 6, and 10, respectively. As shown in Figure 2, specaccum species accumulation curve reaches the plateau, indicating the depth for sequencing was sufficient.

Microflora Diversity The α -diversity difference for the four groups is shown in Table 7. Chao1 Index and ACE

Index were used to evaluate the actual number of species in the sample. The Shannon Diversity Index comprehensively considered the richness and evenness of the community. Simpson Diversity Index is also one of the commonly used indices to evaluate community diversity. No significant difference was found in α -diversity of intestinal microflora of broilers among treatment groups ($P > 0.05$). The results of a PCA analysis and NMDS analysis are shown in Figures 3 and 4.

Relative Abundance of Intestinal Microflora at the Phylum Level The effects of different treatments on the relative abundance of intestinal microflora of broilers at the phylum level are shown in Table 8. At the phylum level, particle size had no effect on the structure of intestinal microflora of broilers ($P > 0.05$).

Relative Abundance of Intestinal Microflora at the Family Level The data on the relative abundance of intestinal microflora of broilers at family level are shown in Table 9. The relative abundance of *Lactobacillaceae* in the C/C/C group was significantly decreased ($P < 0.05$), whereas the relative abundance of *Peptostreptococcaceae* was significantly increased ($P < 0.05$). Compared with the F/C/C and C/F/F groups, the F/F/F group significantly increased the relative abundance of *Moraxellaceae* ($P < 0.05$).

Relative Abundance of Intestinal Microflora at the Genus Level The effects of different treatments on the

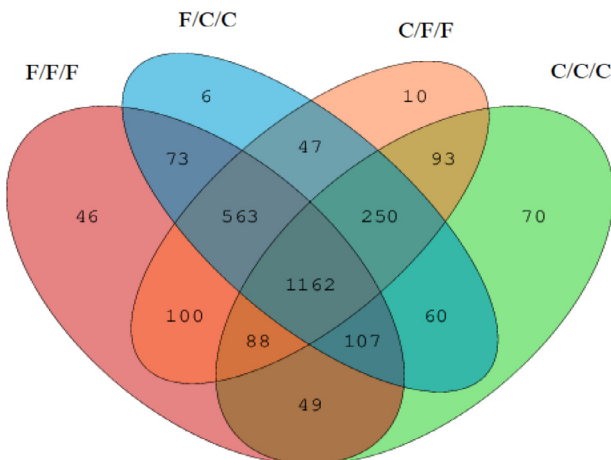


Figure 1. Venn diagram analysis.

Table 7. Effect of different treatments on α -diversity of ileum microorganisms in broilers at d 38.

Items	Group				SEM	P-value
	F/F/F	C/C/C	F/C/C	C/F/F		
Simpson	0.77	0.86	0.84	0.81	0.02	0.390
Chao1	892.39	826.18	895.81	768.51	35.57	0.554
ACE	912.24	834.06	907.13	771.12	36.41	0.723
Shannon	4.70	5.38	5.19	4.67	0.2	0.896

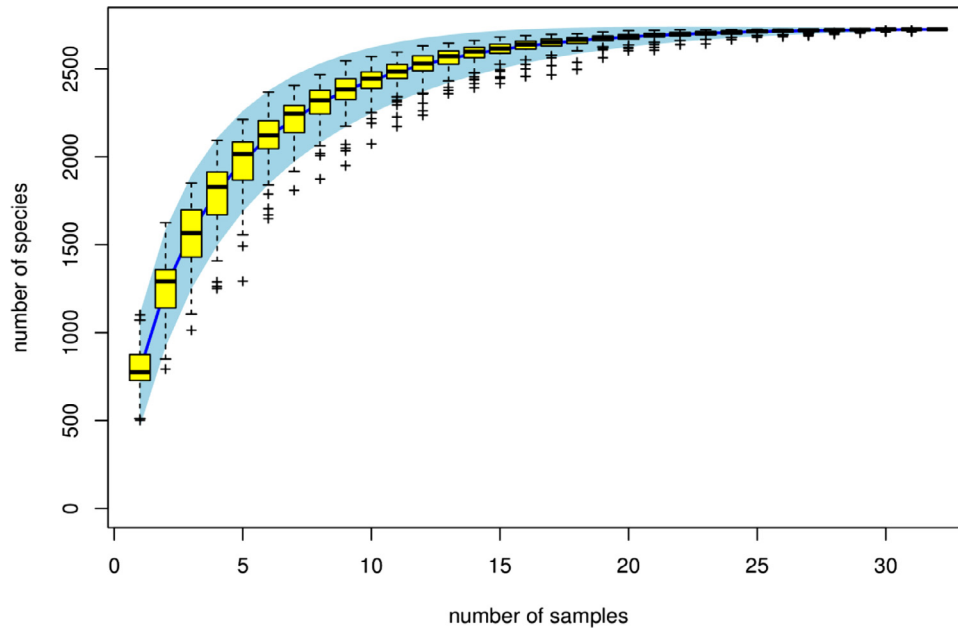


Figure 2. Species accumulation curve of speccacum.

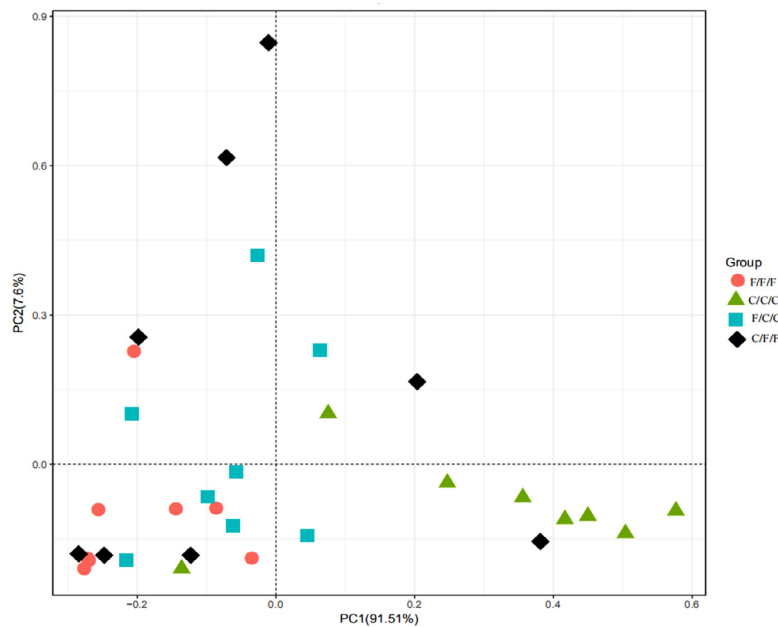


Figure 3. PCA analysis of ileum microorganism. PCA, principal component analysis.

relative abundance of intestinal microflora at the genus level of broilers are shown in Table 10. *Lactobacilli* in the C/C/C group were significantly decreased ($P < 0.05$), while *Acinetobacter* in the F/F/F group was significantly increased ($P < 0.05$).

DISCUSSION

Corn Particle Size on Bird Performance

Grain particle size plays an important role in feed processing. Nir et al. (1995) suggested that bigger particles slowed down digesta passage in the small intestine, resulting in more peristaltic movements and a better

utilization of the nutrients. As grind particle size increases, the surface area of feed will decrease, which will influence nutrient digestion but may promote feeding intake and enhance gut health in broilers (Ge et al., 2017). While Nir et al. (1994a) reported that broiler chickens preferred diets containing particles to those containing fine particles. But it was not clear whether a coarse feed was beneficial for all ages of birds.

The results from this study clearly demonstrated that finely ground corn improved body weight and FCR of broilers during the starter period (d 1–13), whereas coarsely ground corn was more beneficial from the grower phase (d 14–23) to the finisher phase (d 24–37) in terms of body weight, feed intake and FCR. These results are in

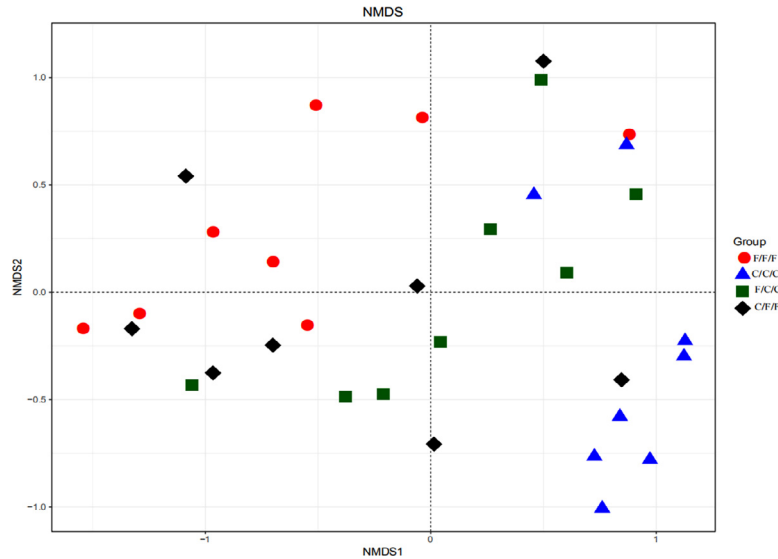


Figure 4. NMDS analysis chart of ileum microorganism. NMDS, nonmetric multidimensional scaling.

Table 8. Effect of different treatments on relative abundance of ileal at the phylum level microbiota in broilers at d 38.

Items	Group				SEM	P-value
	F/F/F	C/C/C	F/C/C	C/F/F		
Firmicutes%	96.54	98.09	93.59	89.33	1.370	0.111
Proteobacteria%	3.38	1.83	6.32	10.54	1.371	0.113
Actinobacteria%	0.026	0.008	0.044	0.045	0.008	0.368
Cyanobacteria%	0.032	0.002	0.010	0.050	0.008	0.149
Bacteroidetes%	0.006	0.039	0.010	0.010	0.006	0.250

agreement with the findings of other studies. For instance, [Chewning et al. \(2012\)](#) compared ground corn to either a particle size of 600 μm (coarse) or a particle size of 300 μm (fine) for their effects on the body weight of broilers. They found marked differences during the starter (d 1–14) and grower (d 15–21) phase favoring the fine particle size, while the difference disappeared during the finisher phase. [Ge et al. \(2017\)](#) showed similar

results with a finer corn particle size in the starter and growth phase and a coarser one later in the phase were beneficial for body weights. [Zang et al. \(2009\)](#) showed that coarse significantly increased ADFI during overall period than fine in mash or pellet diets. The reason for this result may be the coarse ground feedstuff stimulates gizzard development and encourages feeding behavior, leading to improved broiler growth performance.

Gastrointestinal Tract Development and Carcass Yields of Broilers

[Shi et al. \(2017\)](#) reported where the half-eviscerated percentage and eviscerated percentage were not affected when broilers were fed diets with corn ground in 3 different particle sizes, that is, 232 μm, 319 μm, and 380 μm, although carcass yield tended to increase with increasing corn particle size. Similarly, [Rezaeipour and Gaz-](#)

Table 9. Effect of different treatments on relative abundance of ileal microbiota at the family level in broilers at d 38.

Items	Group				SEM	P-value
	F/F/F	C/C/C	F/C/C	C/F/F		
Lactobacillaceae%	89.30 ^a	38.93 ^b	74.55 ^a	70.29 ^a	3.255	<0.001
Peptostreptococcaceae%	5.82 ^b	52.54 ^a	15.07 ^b	15.36 ^b	4.280	<0.001
Enterobacteriaceae%	2.77	1.59	6.13	7.75	1.193	0.242
Clostridiaceae%	0.70	3.62	1.89	2.84	0.449	0.116
Mogibacteriaceae%	0.61	0.74	1.69	0.54	0.187	0.095
Moraxellaceae%	0.26 ^a	0.11 ^{ab}	0.05 ^b	0.02 ^b	0.031	0.029

^{ab}Within a line, numbers with different superscripts differ statistically at $P < 0.05$.

Table 10. Effect of different treatments on the relative abundance of ileal microbiota at the genus level in broilers at d 38.

Items	Group				SEM	P-value
	F/F/F	C/C/C	F/C/C	C/F/F		
Lactobacillus%	89.30 ^a	38.93 ^b	74.55 ^a	70.29 ^a	3.26	<0.001
Candidatus_Arthromitus%	0.20	0.53	0.40	0.29	0.09	0.655
Acinetobacter%	0.37 ^a	0.11 ^b	0.05 ^b	0.02 ^b	0.04	0.014
Turcibacter%	0.003	0.136	0.120	0.008	0.03	0.241

^{ab}Within a line, numbers with different superscripts differ statistically at $P < 0.05$.

ani (2014) found that different particle sizes of feed did not affect the relative weight of breast and thigh. However, Arun et al. (2014) found that different feed particle sizes can affect the slaughter performance of broilers. Our study also found the carcass yield and thigh relative weight were significantly increased when broilers fed fine ground corn from 1 to d 13 and coarse ground from d 14 to 37. Such discrepancies are not surprising because the corn particle sizes in our study were much larger, ranging from 485 μm for the finely ground corn to 2 409 μm for the coarsest particle size.

The influence of particle size on gut development is akin to the effect of structural components (Hetland et al., 2005), where a certain degree of physical coarseness is required in order to have a discernable positive impact on gut health (Choct, 2009). We have found that coarsely ground corn had significant positive impacts on gut development. Thus, the relative weights of the gizzard, duodenum, jejunum and ileum were improved by diets containing coarsely ground corn compared with those containing finely ground corn. These results are similar to the other studies. Merely from a gut development's point of view, feeding starter chicks with coarsely ground corn can be beneficial. Thus, Nir et al. (1994b) found that when one-day-old chicks were fed coarse and medium coarse ground corn, the gizzard weight was increased by 26 to 41% when compared with chicks fed fine ground corn. The development of the proventriculus and gizzard was influenced by grain particle size, with coarse ground grain enhancing their development (Jones and Taylor, 2001; Jacobs et al., 2010; Arun et al., 2014). Shi et al. (2017) found that the coarsely ground feed tended to increase the relative weight of the duodenum and jejunum, and significantly improved the relative weight of the ileum at the finisher phase.

The current study showed that carcass indices were not influenced by corn particle size during the starter period. But coarsely ground corn increased the relative weights of thigh and breast. These results seemed to suggest that diets that were conducive for digestive organ development, in general, improved the processing yields of certain carcass cuts, such as breast meat yield. Indeed, large digestive organs with concomitant positive changes in the micro structure of the intestine in chickens not only enhance gut health but also maintain good bird performance and carcass yield (Sittiya et al., 2020).

Ileal Microflora

The intestinal microbiota is an integral part of animal health (Antonissen et al., 2016) and its population and diversity are closely related to diet (Luo et al., 2017). In our study PCA and NMDS analysis showed obvious differences due to corn particle size. In the PLS-DA analysis, each treatment was represented by the same colored dots. The farther the distance between the dots in different groups is, the better the classification model. In the current study, the microbiota structure between the control (fine particle size for the entire period), C/F/F

(coarse particle size for the starter fine particle size for the grower and finisher period) was similar, whereas it differed widely among the control (fine particle size for the entire period) and C/C/C group (course particle size throughout the study duration).

At the phylum level, Firmicutes, Proteobacteria, and Cyanobacteria are the most abundant bacteria in the ileal microflora of broilers (Xiao et al., 2017). Firmicutes have been reported to improve FCR and growth performance of livestock and poultry (Zhui et al., 2017). Proteobacteria is the major phylum of Gram-negative bacteria and it contains many pathogenic organisms such as *Escherichia coli* and *Helicobacter pylori* (Liu, 2017). The current study, the most abundant bacteria at the phylum level are Firmicutes, Proteobacteria and Cyanobacteria. Corn particle size did not have a significant effect on the structure of intestinal flora at the phylum level. At the family level, studies have shown that Lactobacillaceae are a family of lactic acid bacteria which are open thought to be beneficial in inhibiting the activity of pathogenic microorganisms in the intestine, leading to improved intestinal health of broilers (Lan et al., 2004). Grozina (2014) also found that the abundance of intestinal Lactobacillaceae was increased concomitantly with the growth performance when antibiotics and probiotics were added to broiler diets. Peptostreptococcaceae (Costa et al., 2017) have different cell morphologies, including spherical, rod-shaped, and filamentous, which were all anaerobic bacteria found mostly in animal body cavities, feces, soil, and humus sediments. The number of Peptostreptococcaceae increased during purulent infection (Galperin et al., 2016). Costa et al. (2017) showed that the abundance of Peptostreptococcus was significantly reduced in the gut of broilers whose performance was improved by antibiotics. *Moraxella*, one of the common respiratory pathogens (Pierre et al., 2019), causes lower respiratory tract infections, and its abundance is second only to *Haemophilus influenzae* and *Streptococcus pneumoniae* (Yang, 2014).

The results of this study showed that Lactobacillaceae and Peptostreptococcaceae were the most abundant bacteria at the family level. Coarsely ground corn fed through the study duration reduced the abundance of Lactobacillaceae compared with other treatments as shown in Table 10 and depicted in Figure 1. There is ample evidence suggesting that the physical characteristics of diet can have profound effect on gut development (Choct, 2009; Xu et al., 2017). For instance, coarse ground or the use coarse fibre additives positively affects gizzard function and general gut development in poultry (Zaefarian et al., 2016). On the other hand, fine particles offer more surface area, and when presented to animals, the particles may enter the respiratory system through inhalation and stimulate the nasal mucosa, exposing the animal to increased the risk of respiratory pathogens (Xing et al., 2016). In our study, birds fed the control diet enriched the abundance of Moraxellaceae. The family of *Moraxellaceae* contains many species that are harmless although some species that may cause infections through their colonization of mucosal membranes in animals. At

the genus level, our study showed that the abundance of *Acinetobacter* was demonstrably enriched in birds fed the control diet, which was used the finely ground corn through the study period. As an opportunistic pathogen, *Acinetobacter* may increase the chance of pneumonia and bacteremia (Wang et al., 2019), it may also induce broiler respiratory diseases, and cause symptoms such as diarrhea, which will increase the mortality during broiler breeding (Wani et al., 2006).

In addition, finely ground grain has adverse effects on the development of the gastrointestinal tract and the morphology of intestinal villi of broilers (Dahlke et al., 2003). It can be inferred that the fine ground corn fed at d 1 to 37 affected the normal development of the gut, leading to the proliferation of *Acinetobacter*.

CONCLUSION

Feeding finely ground corn during d 1 to 13 and coarsely ground corn during d 14 to 23 and d 24 to 37 can improve growth performance, carcass yield, leg weight, and increase the abundance of *Lactobacillus* and decrease the abundance of *Peptostreptococcus* and *Acinetobacter* in the ileum of broilers.

An appropriate grind size of the corn component of broiler diets is important for gut health and performance. In general, finely grinding corn for the starter diet followed by the use of coarsely ground corn improved bird performance and carcass yield, enhanced gut development and promoted beneficial microflora in broilers raised in cages.

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There was no real or perceived conflict of interest by the scientists authored this paper.

REFERENCES

- Abdollahi, M. R., F. Zaefarian, H. Hunt, M. N. Anwar, and V. Ravindran. 2019. Wheat particle size, insoluble fibre sources and whole wheat feeding influence gizzard musculature and nutrient utilisation to different extents in broiler chickens. *J. Anim. Physiol. An. N.* 103:146–161.
- Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Influence of particle size on the performance, digesta characteristics and energy utilisation of broilers fed maize and wheat based diets. *Poult. Sci.* 86:2615–2623.
- Antonissen, G., V. Eeckhaut, K. Van Driessche, O. Loncke, H. Freddy, D. Richard, J. M. Robert, and V. I. Filip. 2016. Microbial shifts associated with necrotic enteritis. *Avian. Pathol.* 45:308–312.
- Arun, R. U., B. Chacko, K. Narayanankutty, and S. Harikrishnan. 2014. Effect of feed particle size on slaughter parameters in broiler chicken. *I. J. S. R.* 3 492–492.
- Aviagen. 2014. Ross 308 Broiler. Performance Objectives. <https://www.winmixsoft.com/files/info/Ross-308-Broiler-PO-2014-EN.pdf>. Accessed May 2022.
- Chewning, C. G., C. R. Stark, and J. Brake. 2012. Effects of particle size and feed form on broiler performance. *J. Appl. Poult. Res.* 21:830–837.
- Choct, M. 2009. Managing gut health through nutrition. *Brit. Poult. Sci.* 50:9–15.
- Costa, M. C., J. A. Bessegatto, A. A. Alfieri, J. S. Weese, and J. A. Filho. 2017. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. *Plos. One* 12:e0171642.
- Dahlke, F., R. Aml, A. M. Kessler, A. R. Lima, and A. Maiorka. 2003. Effects of corn particle size and physical form of the diet on the gastrointestinal structures of broiler chickens. *Braz. J. Poult. Sci.* 5:61–67.
- Galperin, M. Y., V. Brover, I. Tolstoy, T. Igor, and Y. Natalya. 2016. Phylogenomic analysis of the family Peptostreptococcaceae (Clostridiumcluster XI) and proposal for reclassification of *Clostridium litorale*(Fendrichet al.1991) and *Eubacterium acid aminophilum* (Zindelet al.1989) as *Peptoclostridium litorale* gen. nov. comb. nov. and *Peptoclostridium acidaminophilum* comb. nov. *Int. J. Syst. Evol. Micr.* 66:5506–5513.
- Ge, C. Y., J. Li, H. Duan, J. B. Yu, Z. Yu, J. Sun, and Y. Qin. 2017. Effects of different diet particle size combinations on growth performance of broilers in early and later periods. *Chin. J. Anim. Nutr.* 29:4342–4348.
- Grozina, A. A. 2014. Gut microbiota of broiler chickens influenced by probiotics and antibiotics as revealed by T-RFLP and RT-PCR. *Agric. & Biol.* 6:46–58.
- Healy, B. J., J. D. Hancock, P. J. Bramel-Cox, K. Behnke, and G. A. Kennedy. 2010. Optimum particle size of corn and hard and soft sorghum grain for nursery pigs and broiler chicks. *Kansas. Agric. Exp. Res. Rep.* 10:1968–2014.
- Hetland, H., B. Svihus, and M. Choct. 2005. Role of insoluble fiber on gizzard activity in layers. *J. Appl. Poult. Res.* 14:38–46.
- Jacobs, C. M., P. L. Utterback, and C. M. Parsons. 2010. Effects of corn particle size on growth performance and nutrient utilization in young chicks. *Poult. Sci.* 89:539–544.
- Jones, G. P. D., and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. *Brit. Poult. Sci.* 42:477–483.
- Kheravii, S. K., R. A. Swick, M. Choct, and S. B. Wu. 2017. Coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens. *Poult. Sci.* 96:3272–3281.
- Lan, P. T. N., M. Sakamoto, and Y. Benno. 2004. Effects of two probiotic *Lactobacillus* Strains on Jejunal and cecal microbiota of broiler chicken under acute heat stress condition as revealed by Molecular Analysis of 16S rRNA Genes. *Microbiol. Imm.* 48:917–929.
- Liu, G. 2017. L-Glutamine and L-arginine protect against enterotoxigenic *Escherichia coli* infection via intestinal innate immunity in mice. *Amino. Acids* 49:1–10.
- Luo, Y. H., Z. Ling, L. Hua, S. Hauke, A. G. Wright, K. Zhang, X. Ding, Q. Zeng, S. Bai, and J. Wang. 2017. Different types of dietary fibers trigger specific alterations in composition and predicted functions of colonic bacterial communities in BALB/c Mice. *Front. Microbiol.* 8:966–980.
- Moran, E. T. Jr 1982. Comparative nutrition of the fowl and swine. Page 250 in *Gastrointestinal Systems*. The ET Moran, ed. University of Guelph, Guelph, Canada.
- Nir, I., G. Shefet, and G. Aroni. 1994a. Effect of particle size on performance. 1. Corn. *Poult. Sci.* 73:45–49.
- Nir, I., R. Hillel, G. Shefet, and Z. Nitsan. 1994b. Effect of grain particle size on performance. 2. Grain texture interactions. *Poult. Sci.* 73:781–791.
- Nir, I., R. Hillel, and I. Ptichi. 1995. Effect of particle size on performance: 3. Grinding pelleting interactions. *Poult. Sci.* 74:771–783.
- Pierre, V. D., G. Leroux-Roels, G. Leroux-Roels, I. De Ryck, A. Tasciotti, M. Dozot, L. Moraschini, M. Testa, and A. K. Arora. 2019. Safety and immunogenicity of non-typeable *Haemophilus influenzae*-Moraxella catarrhalis vaccine. *Vaccine* 37:3113–3122.
- Putra, A., D. Ismail, and N. Lubis. 2018. Technology of animal feed processing (fermentation and silage) in bilah hulu village, labuhan batu regency. *J. Saintech. Transfer.* 1:41–47.
- Rezaei-pour, V., and S. Gazani. 2014. Effects of feed form and feed particle size with dietary L- threonine supplementation on performance, carcass characteristics and blood biochemical

- parameters of broiler chickens. *J. Anim. Sci. Technol.* 56:20–25.
- Shi, Z. Y., H. C. Deng, M. Wang, Q. S. Guo, J. H. Zheng, J. S. Situ, G. M. Xie, X. Y. Yang, and W. J. Shen. 2017. Effect of corn particle size on performance and gastrointestinal indices of broilers. *Chin. J. Anim. Nutr.* 29:75–79.
- Sittiya, K., J. Yamauchi, W. Nimanong, and N. Thongwittaya. 2020. Influence of levels of dietary fiber sources on the performance, carcass traits, gastrointestinal tract development, fecal ammonia nitrogen, and intestinal morphology of broilers. *Braz. J. Poult. Sci.* 22:1–8, doi:10.1590/1806-9061-2019-1151.
- Wang, T., C. Victoria, S. G. Jenkins, B. J. Hartman, and L. F. Westblade. 2019. *Acinetobacter radioresistens* infection with bacteremia and pneumonia. *Idcases* 15:e00495.
- Wani, S. A., I. Samanta, M. T. Bandey, and M. A. Bhat. 2006. Isolation of *acinetobacter iwoffii* from broiler chicken with septicaemia in Kashmir Valley. *Indian. J. Anim. Res.* 40:61–63.
- Xiao, Y., J. Chen, K. Li, J. Chen, and Y. Hua. 2017. Microbial community mapping in intestinal tract of broiler chicken. *Poultry. Sci.* 96:1387–1393.
- Xing, Y. F., Y. H. Xu, M. H. Shi, and Y. X. Lian. 2016. The impact of PM_{2.5} on the human respiratory system. *J. Thorac. Dis.* 8:E69–E74.
- Xu, Y., Y. M. Lin, C. R. Stark, P. R. Ferket, C. M. Williams, and J. Brake. 2017. Effects of dietary coarsely ground corn and 3 bedding floor types on broiler live performance, litter characteristics, gizzard and proventriculus weight, and nutrient digestibility. *Poul. Sci.* 96:2110–2119.
- Yang, X. 2014. *Moraxellaceae*. *Encycloped. Food Microbiol.* 1:826–833.
- Zaefarian, F., M. R. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. *World's Poul. Sci. J.* 72:277–290.
- Zang, J. J., X. S. Piao, D. S. Huang, J. J. Wang, and X. Ma. 2009. Effects of feed particle size and feed form on growth performance, nutrient metabolizability and intestinal morphology in broiler chickens. *Asian. Austral. J. Anim.* 22:107–112.
- Zhui, L., W. Wang, D. Liu, Y. Guo, and S. B. Wu. 2017. Effects of *Lactobacillus acidophilus* on gut microbiota composition in broilers challenged with *Clostridium perfringens*. *Plos. One* 12:1945–1954.